

## EFFECT OF COW TRAFFIC TYPE ON AUTOMATIC MILKING SYSTEM PERFORMANCE IN DAIRY FARMS

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### ABSTRACT

The performance of automatic milking system (AMS) units on three farms with free and directed cow traffic was examined in this study. Farm A housed 123 Holstein cows and used free traffic. Farms B and C housed 104 and 102 Holstein cows, respectively, and both used milk-first-cow traffic. Daily milking frequency per cow was highest on Farm B (2.85), followed by Farm C (2.69), and Farm A (2.36). Although the daily milking rate per cow was lowest on Farm A, the daily milking yield was higher than those on the other two farms (Farm B, 25.30 kg; Farm C, 24.33 kg) with 27.33 kg. The number of daily refusals was significantly higher on Farm A (34.93) compared to refusals on the other two farms ( $P < 0.05$ ). The hourly milking capacities of the two AMS were lowest throughout the day on Farm A (4.4 visits), followed by Farms B and C, with 6.5 and 7.2 visits, respectively. AMS loading was higher on Farm A (78.09%) than on the other farms, which reduced the time wasted on Farm A by 15.23%. Most cows were milked two to four times daily on Farms B and C and one to two times daily on Farm A. A cow-traffic system must be chosen at the beginning of the investment. No traffic type is inherently better than any other. Farmers must be able to choose the appropriate cow traffic system for their farm, lifestyle, and labor qualification.

**Key words:** Robotic milking, automatic milking system (AMS), cow traffic system, milking capacity, robotic load, milk yield, milking frequency.

### INTRODUCTION

It is necessary to carry out studies on automatic milking system (AMS) technology. These systems are expensive, and very little is known regarding the effects of the interactions with technology and employees on AMS performance. The basic principle in AMS technology is the initiation of milking by the cow, which comes to the AMS voluntarily at any time of day. The cow is expected to instinctively initiate milking as it does feeding, drinking water and resting behavior (Ketelaar-de Lauwere 1999). The AMS is a very different than conventional milking system. The cow's access to the feed line may sometimes decrease on AMS farms with free traffic. In directed systems, the cow is encouraged to pass through the milking stall to reach the feed line, which may reduce feeding time and frequency (Rodriguez 2012; Unal and Kuraloglu 2015).

Milking, milk yield, milk quality, milking frequency, cow traffic, behaviors and coping with environmental issues are the main components of AMS that should be examined (Brouček and Tongel' 2015). In this system, cows wait in front of the milking stall to be milked. Cows are thus prevented from going to the feed line and waste time standing in the waiting area instead of resting, which also causes them to foot and nail problems. All these factors reduce total milk yield and farm income (Sonck 1996; Stefanowska *et al.* 1999).

The additional milk production from cows milked more than twice in a day is important to determine. The amount of milk to be obtained in three milkings may increase by 20% on average compared to two milkings. Four milkings may provide an additional increase between 5 and 10%. The additional milk yield provided by stimulating milk-producing cells is associated with better feeding and better management on farms where three milkings are performed (de Koning and Ouweltjes 2000; Hobbis 2013).

In AMS, each cow is milked between two and four times a day. Many factors such as feeding method, cow flow, AMS performance and herd size affect milking frequency (Ipema 1997; Halachmi 1999). Cows are milked an average of 2.4 and 2.8 times in a day in free-traffic systems and an average of 2.5 and 2.9 times a day in directed-traffic systems (Ipema 1997; de Koning and Ouweltjes 2000). These figures are affected by factors other than cow traffic. The necessity of fetching cows and involuntary milking should also be considered (Rodriguez 2012 and 2013). Castro *et al.* (2012) found the annual milk yield of an AMS to be 549,734±126,432 kg/year despite the 1947±978 h/year wasted by 53-62 cows, with a daily milking average per cow of 2.41 and 2.97 times/day. These studies were carried out in countries where automatic milking systems are commonly used. However, the differences arising from the unique characteristics of dairy farms in different countries may affect the results.

The high purchase cost of an AMS unit requires that the robot be used at the highest possible capacity. The daily milking frequency of each cow is one of the main factors affecting the full-capacity loading of the robot (Ketelaar-de Lauwere, 1999; Bach *et al.*, 2009). Thus, the underlying factors are the cow's willingness to enter the AMS, ease of access to the robot, provision of concentrated feed in the AMS, the tendency of cows with high yield to be milked more frequently, milking period (influenced by milk yield and milk flow rate), discharge of the milk-cooling tank (influenced by the time required to wash the milk line and the presence of a spare milk tank in the system) (Laurs *et al.* 2009; Priekulis and Laurs 2012; Anonymous 2013).

This study aimed to determine AMS performance on three farms, one of which used free traffic and the other two used milk-first traffic. The main criteria examined in the study were the daily milking frequency per cow, daily milk yield per cow (kg/cow), milk yield per milking (kg/milking), milk flow rate per cow (kg/min cow), the time spent by the cow in the AMS for milking (min/milking), daily milking interval per cow (h/cow day), the number of daily milkings per AMS, the herd size, the number of daily milkings per AMS, the frequency with which the two AMS were occupied throughout the day, and the time spent milking, washing, idle, and not milking as well as the cow-traffic type.

## MATERIALS AND METHODS

This study was carried out on three farms (A, B and C) where cows were milked by DeLaval brand AMS

(VMS-Voluntary Milking System). Free traffic (Farm A) was used on one of the farms, and milk-first traffic (Farms B, C) was used on the other two farms.

On farm A, an average of 123 Holstein-Friesian cows were in the barn during the study. On this farm, the annual milk yield per cow was approximately 9950 kg. Cows were free to go to the feed line or rest in stalls after they were milked in the AMS. There was no waiting area before the AMS. There were 108 stalls in the resting area. On the farm, the 35 kg total daily feed ration per cow included the following ingredients: 1 kg straw, 4.5 kg alfalfa, 24 kg corn silage, 0.5 kg vitamin and mineral mixture and 5 kg concentrated milk feed. The total mix ration (TMR) was distributed twice a day at 8:00 and 20:00. In addition, concentrated feed was given on an individual basis according to the cow's needs in the milking stall. The feed line was 48 m long on both sides of the barn (feed line width per cow 0.78 m). The cows with delayed milking were brought in for milking four times a day (at 09:00, 15:00, 21:00 and 03:00). There were six troughs in the resting area and feeding area, and four scratching brushes in the feeding area. There was a milk-cooling tank with a 5000 L capacity in the milk house. AMS and the milk line were automatically washed four times a day with alkaline three times and with acid once, respectively (at 00:00, 06:00, 12:00 and 18:00). Each washing lasted for approximately 20 min. The stalls and crossover gates were cleaned twice a day. The manure was taken out of the barn hourly with automatic scrapers. The general layout of the barn on the farm is presented in Fig. 1.

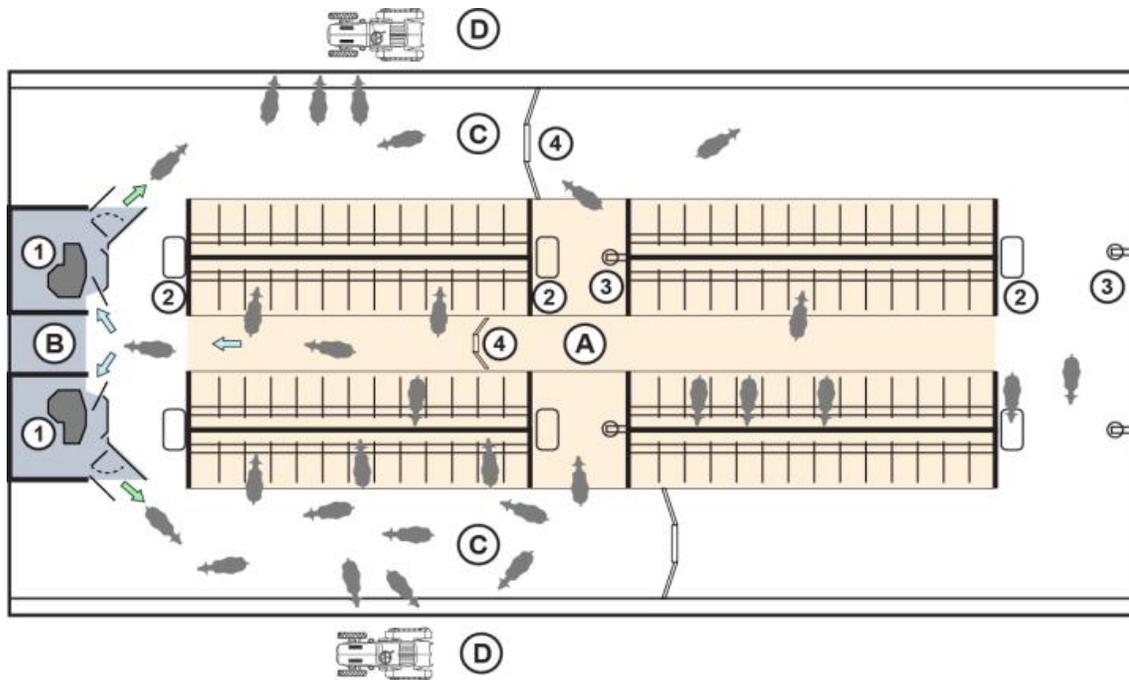
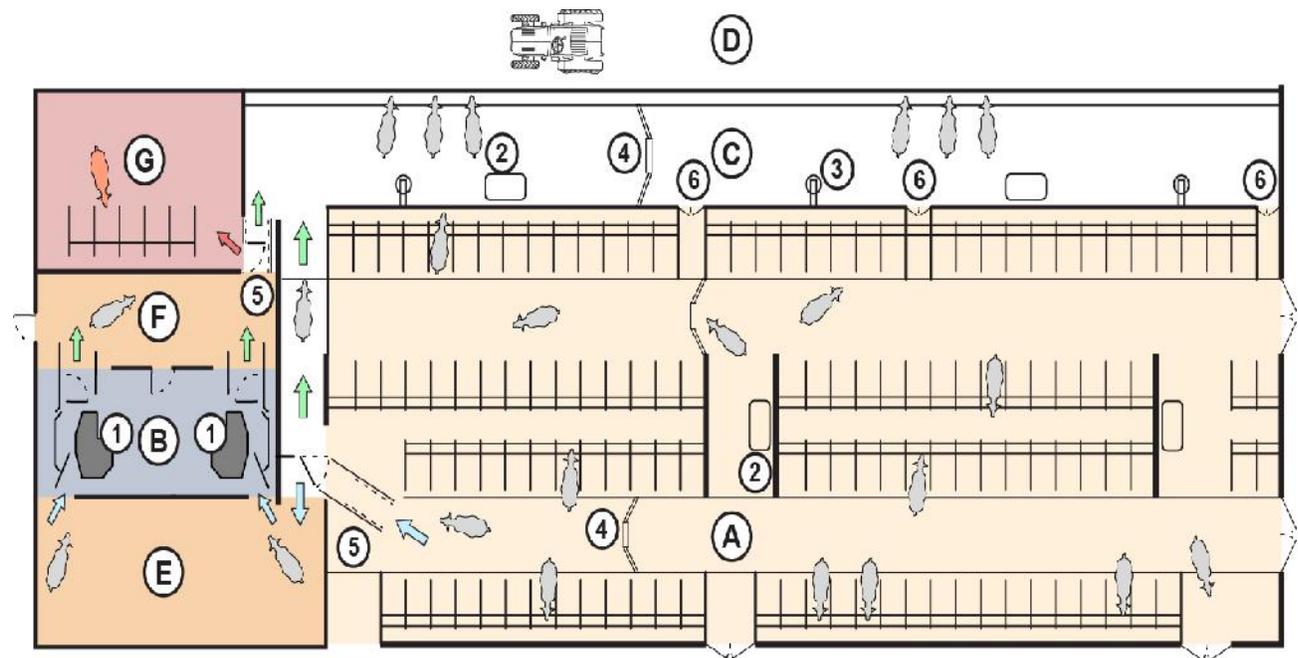


Fig. 1. Layout of barn with free traffic, Farm A

A-Resting area, B-Milking room, C-Feeding area, D-Feeding Line, 1-AMS, 2-Troughs, 3-Brushes 4-Manure scraper

On the second farm (Farm B), the milking process was performed with a total of eight AMS in four barns which were independent of each other. There were two AMS units in each barn. Observations and data were obtained from a barn where two AMS were more actively used. There were an average of 123 Holstein-Friesian cows in this barn during the study. On this farm, the annual milk yield per cow was approximately 9250 kg. The milk-first traffic was used on the farm. A common waiting area was established in front of two AMS access gates. The cow that would go to the AMS from the resting area was directed to the waiting area to enter the AMS by going through the selective gate if it had milking permission; it was directed to the feeding area by the sort gate if it did not have milking permission. The cow passed through a sorting gate that directed it to a separate area (infirmary) if it needed treatment or care after milking and to the feeding area if it did not need treatment or care. The cow could pass from the feeding area to the resting area, where there were stalls, through one-way gates. There were 136 stalls in the barn. There were three one-way gates between the feeding area and resting area. The ration feed was distributed twice a day,

at 8:00 and at 20:00 on Farms A and B. Concentrated feed was additionally given in the feeding station in the AMS according to the individual needs of the cow. The ration feed in the feed line was provided an average of eight times a day. The ration feed distributed at 33 kg per cow consisted of 2 kg straw, 3.5 kg alfalfa, 18 kg corn silage, 0.350 gr vitamin and mineral mixture, 3 kg corn flake, 0.120 kg soda and 6 kg concentrated feed. The feed line was 53 m long on one side of the barn (feed line width per cow, 0.39 m). The cows with delayed milking were brought to milking four times a day (at 09:00, 15:00, 21:00 and 03:00). A total of six troughs were placed in the resting, feeding, waiting areas and separation pen after milking, and three scratching brushes were placed in the feeding area. In the milk house, there was a milk-cooling tank with 9000 L capacity and a buffer tank with 1500 L capacity providing service during washing and milk discharge. AMS were automatically washed three times a day (at 03:00, 12:00, and 21:00) with alkaline three times and acid detergent once, respectively. Each washing stage lasted for approximately 20 minutes. The resting areas, milking waiting area, milking exit area and cross-over gates were cleaned twice a day. The manure was thrown out of the barn hourly with automatic manure scrapers. The layout of the barn of Farm B is presented in Fig. 2.



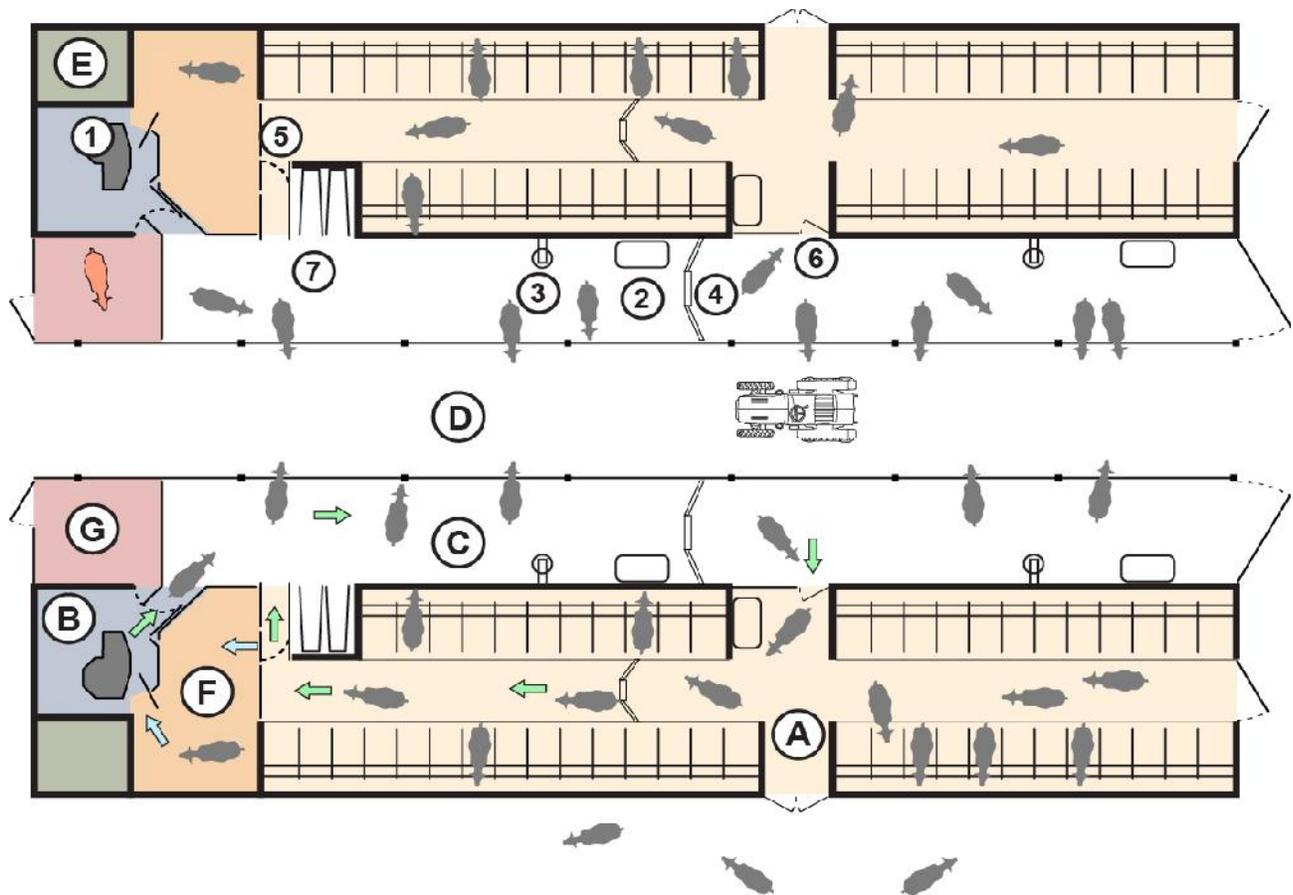
**Fig. 2. The layout of barn with first-milk traffic, Farm B.**

A-Resting area, B-Milking room, C-Feeding area, D-Feeding Line, E-Waiting area, F-Exit area, G-Separation area, 1-AMS, 2-Trough, 3-Brush, 4-Manure scraper, 5-Smart gate, 6-One way gate

On Farm C, unlike on Farm B, milking was performed with AMS in two symmetrical, independent barns. The milking of a total of 104 Holstein-Friesian cows was split equally between two barns. On this farm, the annual milk yield per cow was approximately 8900

kg. Milk-first traffic was used on the farm. A waiting area was established in front of the AMS access gate in both barns. The next cow that would go to the AMS from the resting area was directed to the waiting area and from there to the AMS through the selective gate at its turn. It was directed to the feeding area by the sort gate if it did not have milking permission. The cow came to an exit area after milking in the AMS. Here, the cows passed through a second smart gate. Cows were directed to a separation area through this gate if they had health problems or required any care procedure and directed to the feeding area otherwise. The cow passed from the feeding area to the resting area where there were stalls through one-way gates. There were 50 stalls in each barn section. The feed was distributed twice a day, as on the other two farms. The concentrated feed was additionally given from the feeding station in the AMS according to the individual need of the cow. Unlike on the other two farms, two concentrated feed stations were placed in each barn. The cows with high milk yields were given additional concentrated feed in the AMS and TMR at the

feeding line by the feeding stations. The ration feed (36 kg per cow) consisted of 1 kg straw, 4.5 kg alfalfa, 25 kg corn silage, 0.5 kg compound feed and 5 kg concentrated feed. The feed line was 36 m long on one side of the barn (feed line width per cow, 0.72 m). The cows with delayed milking were brought in for milking four times a day (at 09:00, 15:00, 21:00 and 03:00). In each barn, five troughs were placed in the resting, feeding, waiting area and separation pens after milking and two scratching brushes were placed in the feeding area. In the milk house, there was a milk-cooling tank with 6000 L capacity and a buffer tank with 1500 L capacity providing service during washing and milk discharge. AMS were automatically washed three times a day (at 04:00, 11:00, and 21:00) with alkaline three times and acid detergent once, respectively. Each washing stage lasted for approximately 20 minutes. The resting areas, milking waiting area, milking exit area and crossover gates were cleaned twice a day. The manure was removed hourly with automatic manure scrapers. The layout of the barn of Farm C is presented in Fig. 3.



**Fig. 3. The layout of barn with first-milk traffic, Farm C**

A-Resting area, B-Milking room, C-Feeding area, D-Feeding Line, E-Trimming storage, F-Waiting area, G-Separation area

1-AMS, 2-Trough, 3-Brush, 4-Manure scraper, 5-Smart gate, 6-One way gate, 7-Concentrate Self Feeder (CSF)

The total duration of the study was 60 days. The required data were extracted from a herd-management program. The data were collected from January 28 to March 28 at Farms A and B and from June 1 to July 30 at Farm C. The following basic parameters were examined during the study period:

- The number of daily milkings per cow;
- The daily milk yield per cow (kg/cow day);
- The milk yield per milking (kg/milking);
- The milk flow per cow (kg/min);
- The time spent in the AMS per milking (min);
- The time spent milking per cow each day (h/cow day);
- The daily milking time per AMS and herd size;
- The total time spent milking in the two AMS throughout the day;
- The durations the AMS were occupied in milking, washing, idle and not milking;
- The labor requirement per cow (person h/cow day);
- The type of traffic.

The DeLaval DelPro herd-management program was used on three farms in the study. All data were transferred from the herd program to MS Excel for analysis in a nested design. The research data were transferred daily from the herd program and analyzed in the Minitab 17 program after sorting in MS Excel. The farms were compared using ANOVA and the LSD test ( $P < 0.05$ ). The averages and standard deviations were calculated.

## RESULTS AND DISCUSSION

The number of the cows were milked, the number of days in milking (DIM), number of milkings per cow, daily milk yield per cow, milk yield per milking, milk flow rate per cow, AMS service time per milking, number of daily refusals, milking frequency and labor requirements per cow are presented in Table 1. The daily milking frequency per cow (2.85) was significantly higher on Farm B than on Farms A and C (2.36 and 2.69, respectively) ( $P < 0.05$ ). The values on Farms B and C were between the limit values of the farms with milk-first traffic given in the literature (2.5-2.9) (Ipema 1997; Laurs and Priekulis 2010; Castro *et al.* 2012). The daily milking frequency per cow on Farm A was close to the range of 2.4-2.8 given for free traffic by Ipema (1997). Increasing the AMS visitation frequency by training the cows on Farm A may increase this value to within the acceptable limits. Another solution method would be to fetch the unmilked cows.

The daily milk yields per cow for Farms A, B and C were 27.33, 25.30 and 24.33 kg, respectively (Table 1). Although the number of daily milkings per cow was low on Farm A, the daily milk yield per cow

was high, likely because a greater number of days in the milking (DIM) on Farm A increased the daily milk yield per cow. In addition, the milk yield per milking on Farm A was greater than that on Farms B and C, at 11.59 ( $P < 0.05$ ). No significant difference was observed between the milk yields per milking of Farms B and C ( $P > 0.05$ ). The number of days in milking on Farm A was higher than those on the other two farms, leading to an increase in the milk yield per cow and milking yield. On the other hand, the milking flow rate of the cows on Farm A (1.51 kg/min) was also higher than those on the other two farms. Although the high flow rate on this farm was expected to decrease the time each cow spent in the AMS, the time spent in the AMS was also higher than for the cows on Farms B and C, at 7.69 min (Table 1), which may be explained by the high milk yields of the cows on Farm A. Castro *et al.* (2012) found that the average milk flow rate is 1.44 kg/min, but Heringstad and Kjørten Bugten (2014) determined it to be 1.5 kg/min. Other studies explained milking flow rate only using the automatic system yield (Hogeveen *et al.* 2001; Gäde *et al.* 2007; Carlström *et al.* 2009). The flow rates on Farms B and C may increase with an increase in the number of milking days.

The number of daily refusals (34.93) on Farm A was significantly higher than the numbers on other farms ( $P < 0.05$ ). The absence of pre-milking smart gates on Farm A, where free traffic was used, may be the main reason for refusal. Cows without milking permissions could enter the AMS and occupy a milking stall without being subjected to pre-selection. A pre-selection gate and waiting area can be established on this farm to prevent this problem. On Farms B and C, no statistically significant difference was found in the number of daily refusals ( $P > 0.05$ ). However, the number of daily refusals on Farm B was slightly higher than the number on Farm C (4.02). Factors such as the failure of the AMS arm to attach to the milking head, the fact that a cow that had been just milked could move through the selection gate, and the fact that the AMS could refuse a cow that came in for milking two times in succession and instead leave that cow in the waiting area are potential contributing factors.

The milking interval of 10.28 h/day on Farm A was higher than those of the other farms (Table 1). The cows' willingness to come to the AMS appeared to be low on Farm A, as supported by the low number of daily milkings on this farm (2.36). On the other hand, the free-traffic system on Farm A significantly increased the number of cows that needed to be brought in for milking and therefore increased the labor load. The labor force per cow on Farm A was 0.106 person h/cow day but much lower on Farms B and C (0.050 and 0.043 person h/cow day, respectively) (Table 1).

The number of daily milkings in each AMS unit is presented in Fig. 4. The number of milkings was similar across farms despite the different traffic systems

used on Farms A and B. On these two farms, two AMS were installed in the same barn and each cow was free to select her preferred milking stall. The daily average number of milkings per AMS was 146.5 on Farm A, possibly because there were 21 more cows on Farm B than on Farm A. On Farm C alone, the two AMS units had different numbers of daily milkings (135.6; 143.5), likely because the cows in the two independent barns had different yield profiles. However, all cows on the other two farms were housed in one barn with two AMS, and the cows could select the AMS they preferred. Free choice decreased the difference in the number of milkings between the AMS. In addition, the fact that the AMS were in different barns on Farm C could constitute a disadvantage in the event of mechanical failure. Cows on Farms A and B could be milked in the other AMS in the case of robot failure.

The results showed that the hourly average milking capacities varied throughout the day between 4.4 and 13.8 for Farm A, between 6.5 and 15.1 for Farm B, and between 7.2 and 15.4 for Farm C (Fig. 5). On average, visitation to the AMS on Farm A was highest (12.2 visits/h), followed by Farms B and C (12.1 and 11.6 visits/h, respectively). The AMS was washed four times per day on Farm A and three times per day on Farms B and C. As seen in the figure, the lowest milking capacities seen were 4.4, 6.5 and 7.2, respectively, on Farms A, B and C. The milking capacity on Farm A was much lower than those on the other farms; the milk in the cooling tank was discharged during system washing (between 18.00 and 19.00). The figures for the AMS on Farm A are close because milk transfer required approximately 75 minutes and no buffer tank was available during tank washing. A buffer tank was needed on Farm A to prevent this loss. On the other hand, the capacity of the milk-transfer pump that was used while discharging the milk on Farm A was low. The use of a high-capacity transfer pump that can perform milk discharge during washing can solve this problem. Furthermore, milk transfer can be shifted from 18.00 to 19.00, when many cows come to the AMS for milking, to a period during which milking is less intense. Thus, the length of the milking line that occurs during these times can be reduced. The problems affecting AMS capacity have also been explained in other sources, and similar solutions have been proposed (Hermans *et al.* 2003; Laurs *et al.* 2009; Castro *et al.* 2012; Markey 2013; Gaworski *et al.* 2016).

The ratios of time spent milking, standing idle, washing and not milking on three farms are presented in Fig. 6. Farm A had the greatest AMS loading percentages (78.9%). The greater herd size and time spent fetching cows on Farm A compared to the other two farms are likely contributors. The AMS on Farm C spent the most time idle (30.71%). Although the herd sizes were similar

on Farms B and C farms, the idle time on Farm C was 7.39% higher than that on Farm B. Farm C had fewer daily milkings per cow than did Farm B, increasing the idle time. On the other hand, the AMS on Farm A time spent less time idle (15.23%) than did those on the other two farms, likely because of its greater herd size. Frequent fetching of the cows delays milking but is a requirement of free-traffic systems. In the study carried out by Laurs *et al.* (2009), in a barn with directed traffic and 88 cows, 19% of the time was spent idle. The idle times on Farms B and C, which used milk-first traffic systems, were higher than the values determined by Laurs *et al.* (2009) and Gaworski *et al.* (2016). On Farms B and C, idle time can be reduced by slightly increasing the herd size. On the other hand, increasing herd size is not the only factor that increases AMS performance. Traffic management is also important (Devir *et al.* 1999). It is necessary to fetch and train cows that do not come in for frequent milking to reduce idle time in the AMS.

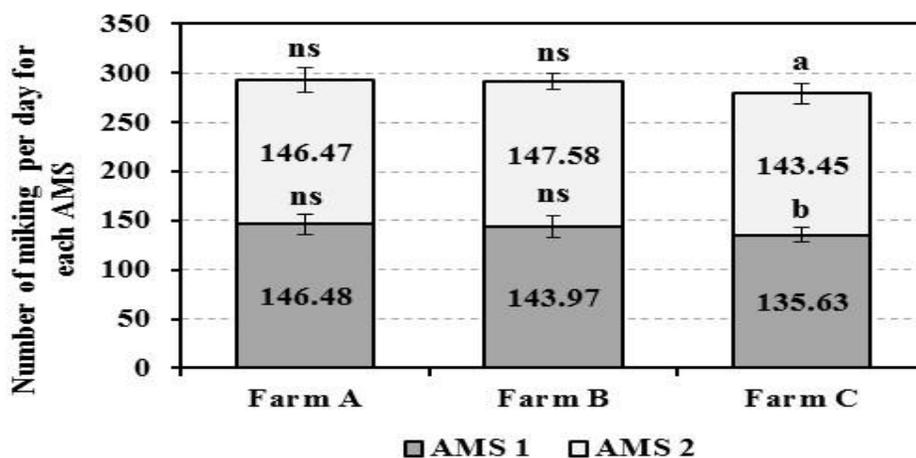
The AMS on Farm A was washed once more per day than were those on the other farms, which increased the time spent washing (4.7%). Times when no milking occurred but the AMS was occupied included 'only feed', 'refusal' and 'without milking'. Therefore, Farm A has the greatest time without milking (1.98%) among the three farms. These numbers were much lower on Farms B and C (0.62% and 0.14%, respectively). Refusals constituted the largest percentage of time without milking on Farm A. The number of refusing cows was kept at a minimum by the smart gates on Farms B and C. Moreover, losses arising from releasing 'unmilked' cows were also determined on all three farms. For this reason, it is necessary to consider cow selection at the beginning of investment in the farm. Rear udders that are not angled or closely placed can reduce times without milking.

The percentages of cows milked relative to the number of daily milkings are given in Fig. 7. As shown in the figure, Farm A tended to milk the cows once or twice a day, while Farms B and C tended to milk the cows two to four times a day. The numbers of average daily milkings (Table 1; 2.36 for Farm A, 2.85 for Farm B and 2.69 for Farm C) may explain the above result. Farm B had the greatest percentage of cows milked two to four times a day (98.11%), while Farm A had the greatest percentage of cows milked one time a day (3.99%). AMS visitation decreased on Farm A, which used free traffic, because the cows preferred the roughage on the feed line to the feed offered in the AMS. This preference also decreased milking frequency (1.36% milked four times a day). The traffic system used on Farms B and C increased AMS visitation by motivating the cow with feed. The number of AMS visits can be increased using appropriate feeding strategies and training and by routine fetching of unmilked cows.

**Table 1. Characteristics of the cow groups**

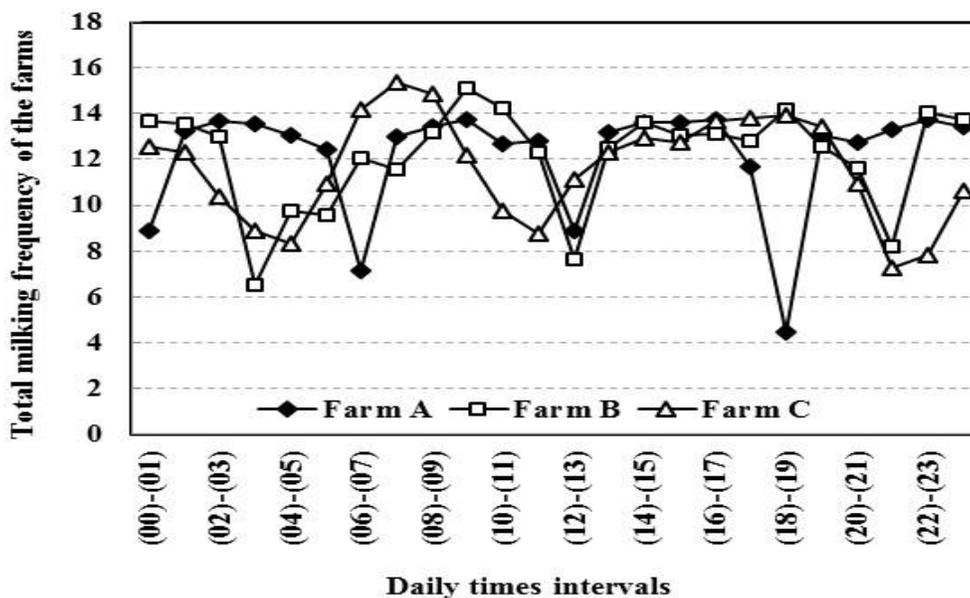
Parameter	Farm A	Farm B	Farm C
The number of cows milked	123	102	104
Day of milking (DIM)	235	185	69
The number of milking per cow per day	2.36±0.15c*	2.85±0.15a	2.69±0.11b
The daily milk yield per cow (kg/cow day)	27.33±1.26a	25.30±1.74b	24.33±1.28c
The milk yield per milking (kg/milking)	11.59±0.75a	9.26±2.04b	9.36±2.57b
The milk flow rate per cow (kg/min)	1.51±0.06a	1.25±0.06c	1.35±0.05b
The time passed in the AMS per milking (min)	7.69±0.35a	7.11±0.27b	6.71±0.30c
The number of daily refusals	34.93±12.64a	4.02±3.46b	1.53±1.34b
Milking interval (h/day cow)	10.28±0.78a	8.60±0.46c	9.09±0.38b
The labor requirement per cow (person h/cow day)	0.106	0.050	0.043

\* The letters of a, b and c refer to the significance of the differences between consecutive values at a minimum level of 95%.



**Fig. 4. The numbers of daily milkings for each AMS on each farm**

The error bar represents the standard error. Different letters indicate significant differences (P<0.05)



**Fig. 5. The distribution of hourly milkings of the two AMS on each farm**

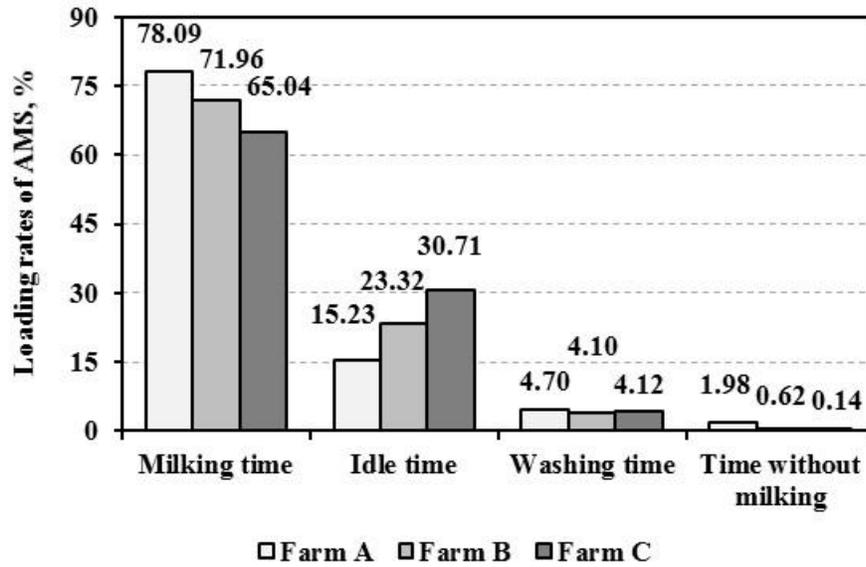


Fig. 6. The percentage of AMS time spent milking, standing idle, washing and performing without milking

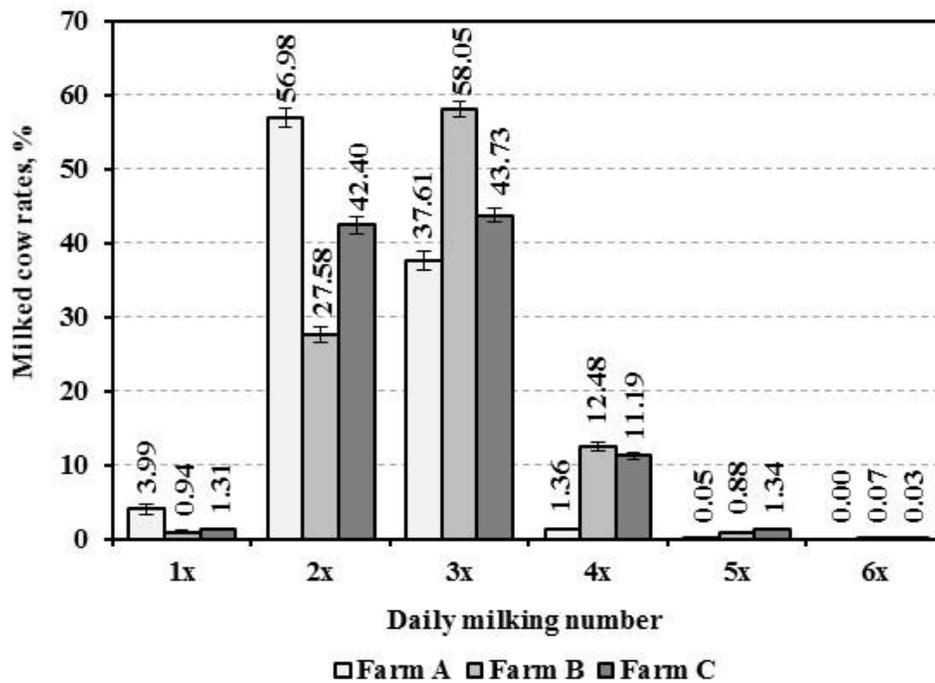


Fig. 7. The distribution of milked cows in relation to the number of daily milkings

**Conclusions:** The factors affecting AMS performance on three farms where free and milk-first traffic were used are summarized below:

- Although the number of daily milkings per cow (2.85 and 2.69, respectively) on Farms B and C, which used milk-first directed traffic, were acceptable, this number (2.36) was low on Farm A. Fetching the cows, training the cows to come to the AMS and using more

appealing feed in the AMS on Farm A could improve the number of visits to the AMS per cow.

- The daily milk yield on Farm A was higher (27.33 kg/day) than that on the other two farms because of the high milk yield per milking (11.59 kg/milking), milking flow rate (1.51 kg/min) and the milking time spent in the AMS per visit (7.69 min).

- The refusal rates were very high (34.93) because there was no smart-gate pre-selection on Farm A, so cows without milking permission were able to enter the AMS and occupy the milking cabin. This number can be reduced by establishing a pre-selection area in front of the AMS.
- Farm A required more labor (0.106 person h/cow day) because of the need to fetch cows in a free-traffic system. Cow training could reduce this requirement.
- Although the number of daily milkings per AMS were similar on Farms A and B (146.5 and 145.8, respectively), Farm A had 21 more cows than Farm B. The number of daily milkings per AMS can be increased by increasing herd size on Farms B and C. On Farm C alone, the two AMS had different numbers of daily milkings (AMS 1: 135.6, AMS 2: 143.5) because the two barns housed cows with different yield profiles. On Farms A and B, by contrast, the cows were all kept in one herd and were free to go to the AMS at any time. A more homogenous herd on Farm C would eliminate this difference.
- On Farm A, additional processing time was required; the milking system was completely stopped during milk discharge because there was no spare storage tank. An additional buffer tank is needed in this operation.
- The greater herd size on Farm A compared to Farms B and C increased the rate of AMS loading (78.09%). Hence, it increased the idle times of the AMS on Farms B and C. Increasing the herd size on these two farms will decrease the idle time ratios.
- Most cows were milked two to four times daily on Farms B and C and one to two times daily on Farm A. It is necessary to take administrative and feed-related measures to increase the number of milkings per cow per day.

**Author's contribution:** H. Unal was the project leader, performed most of the experiments and wrote the paper and corrected. H. Kuraloglu was responsible for experimental and project design. M. Koyuncu and K. Alibas participated in the analysis and discussed the results.

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**Conflict of interest:** Authors have no conflict of interest about publication of this article.

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